Physiology labs for blood (2)

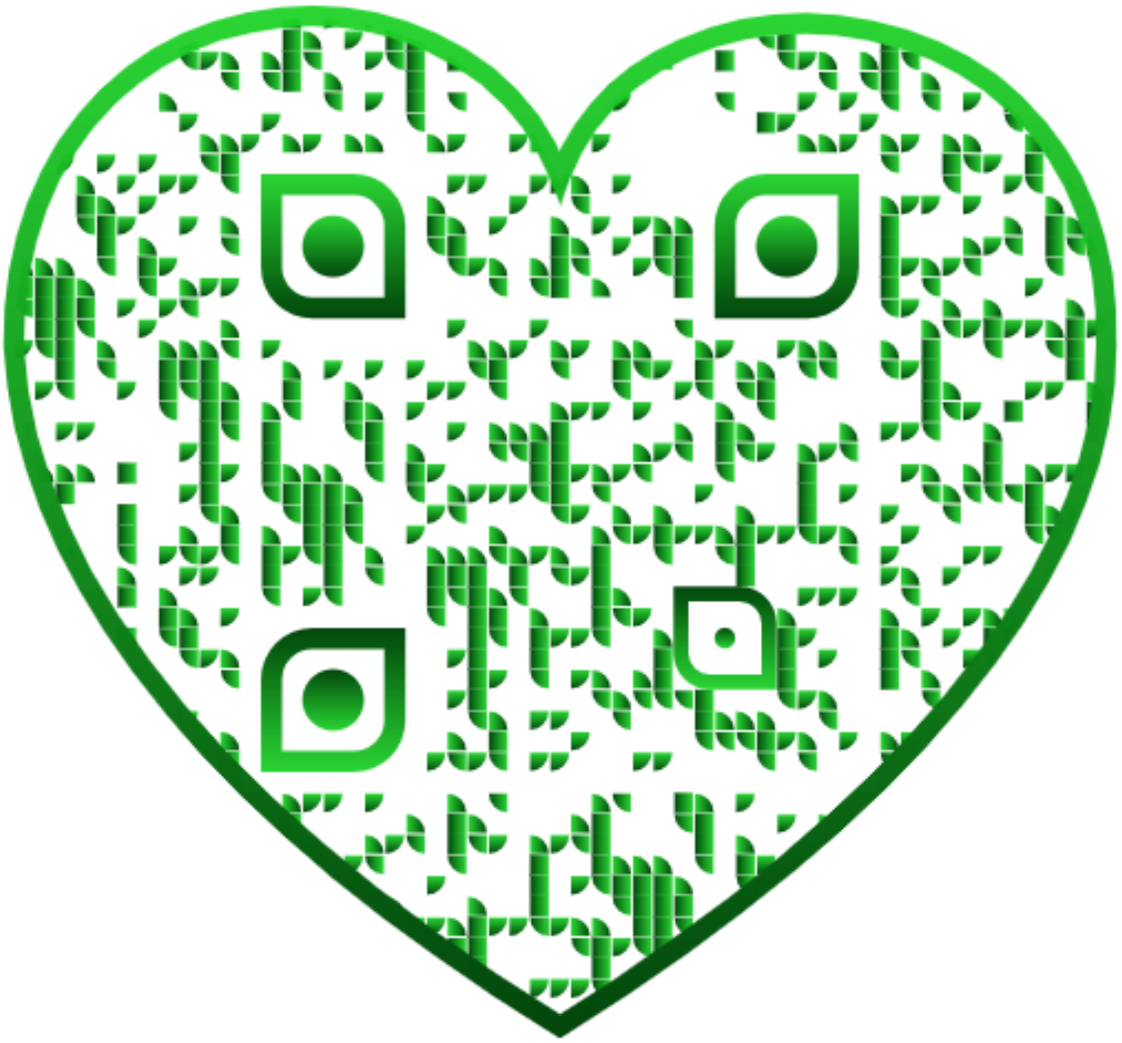
قياس الهيموجلوبين (المعمل الأول)

Hemoglobin estimation (lab 1)

المجموعة السابعة

تلخيص /

أمينة الجيلاني أنس العمراني هيام الجوبي



Hemoglobin(HB) estimation is one of the CBC tests , it gives an overview about the blood by tilling us is the blood having **anemia** or **polycythemia** or it is **normal**

Experience used in HB estimation :-

1- old → manual (sahli method)

2-new → manual

→ automatic

in the first lab we will study the old manual experience **sahli method**

Sahli Method (Acid Hematin method)

this is a **visual method** of Hb estimation, so it is not very accurate. It uses **sahli apparatus**

sahli apparatus characteristics:

1- have a light weight.

2- dose not need electricity.

3- easy to use (user friendly).

4- error percentage = 20% so currently it is not used in the modern laboratories but it is used only in the areas of the world where electricity is not available

Apparatus parts and its reagent:

1- **Sahli graduated tube**: the yellow side of the tube gives the reading in grams of Hb /dl blood while the red side gives it as percentage (14.6 g=100%) .

2- **hemoglobinometer** consisting of the comparator with two standard tubes, we use two standard tubes to reduce the error percentage - the apparatus in the lab are very old and the standard tubes color may have changed , so the result may not be accurate .

3- **Hydrochloric acid "HCL"** as the reagent .

Principle of this method :

The RBCs are destroyed and Hemoglobin is converted into acid hematin by the action of HCL ,The dark brown color of the acid hematin compound is matched visually against a brown glass standard. The dark brown acid hematin color can last for one hour**.**

steps of this method (Technique)

1- Fill the graduated tube with HCL up to the 2 yellow mark .

2- fill the pipette with 20 ul of blood : the blood may be venous blood collected in EDTA anticoagulant or blood obtained by capillary puncture but the latter coagulate fast , so usually we use the EDTA **,** although we only need 20 ul . we collect 2cc of blood to match the blood with the anticoagulant in the EDTA tube

3- Wipe off the blood on the outside of the pipette with a piece of gauze .

4- Empty the pipette into the HCL acid in the tube gently without causing bubbles .

5-mix the acid hematin solution in the tube and allow to stand for 5-10 minutes to make sure that the complete destruction of RBCs and complete conversion of hemoglobin to acid hematin have occurred .

6- Place the tube in the Sahli comparator and start adding HCl or distilled water drop by drop by the dropper . Mix between each addition.

7- continue adding till the color matches that of the standard tubes .

8-Read the volume of solution in the graduated tube, and express as g/dl or as percentage .

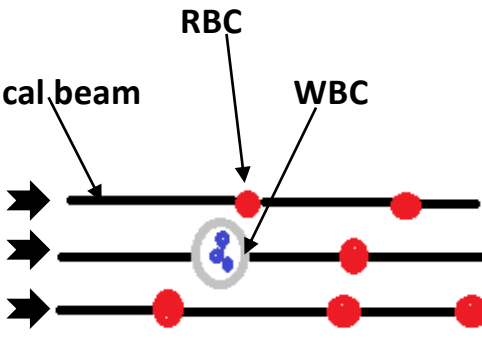
- the normal range for hemoglobin is 13.5 – 17.5 grams for men and 12 – 15.5 grams for women

*** the blood in EDTA tubes can be used within 2 hours , but in laboratories it is stored in refrigerator at 2-8 c^0 for three days**

**** we will study about the new methods in details in the next lab , but now we will talk briefly about the " new automatic method "**

- the new automatic method uses DRABKIN as the reagent

- it gives the CBC count in 30 second to 1 minute



- it works by producing electrical beams , blood cells pass through these beams and cuts it by a certain diameter . for the RBCs the whole diameter is counted as 1 RBC ,but for the WBCs it counts the diameter and the number of the nucleuses of each cell to differentiate between the different types of WBCs . after that the RBCs are mixed with drabkin to convert into colored compound , the absorbance of the colored compound is automatically calculated . finally all the results appear in the screen .

-Note that the new automatic method is not used in the lab & it does not enter in the exam